EFFECTS OF BORON, MANGANESE, COPPER AND ZINC UPON THE GROWTH OF *SETARIA SPHACELATA*

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Abstract

Plants of *Setaria sphacelata* (Schum.) Stapf and Hubbard, Kazungula ecotype, were grown for eight months in sand culture with different levels (0 to 200 ppm) of the micronutrient elements B, Mn, Cu and Zn. Their growth and micronutrient contents were determined.

No definite symptoms of a B deficiency were observed in the main experiment; increased tillering, accompanied by cessation of stem elongation and death of growing points were observed in an accompanying minus-B control in water culture and in a subsequent subculture without B in sand. Symptoms of B toxicity (necrotic spots along leaf margins) and reduced growth were observed with 5 ppm or more of B.

Symptoms of a Mn deficiency—consisting of chlorosis and death of younger leaves and growing points, and eventually death of whole tillers—developed in plants receiving 0·05 ppm or less of Mn. Mn toxicity symptoms (small necrotic spots on older leaves and stems) developed with 100 and 200 ppm of Mn, but growth was not affected.

No definite symptoms of deficiency of Cu or Zn were observed in sand or water culture; growth and development were not affected at low levels of these elements. Symptoms of Cu toxicity developed in plants receiving one ppm or more of Cu; general chlorosis, severely reduced growth and flowering, and often death were noted, particularly at the higher levels of Cu.

Zn toxicity, as indicated by leaf necrosis and severely reduced growth and flowering, developed with 100 and 200 ppm of Zn.

It was concluded that *S. sphacelata* has a definite requirement for Mn and probably also for B. Requirements for Cu and Zn are probably quite low, and contamination apparently was not completely eliminated during this investigation.

Introduction

Although the essentiality of micronutrient elements in plant nutrition has long been established, the responses of many species to these elements have not yet been determined. Native and cultivated grasses in particular have received relatively little attention. This applies even more specifically to African grasses.

Previous research has shown several micronutrients to be indispensable for

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normal growth and development of certain grass species (Sommer, 1927; Schropp and Arendz, 1940; Williams, 1951) under greenhouse conditions. Their effects upon grasses and pasture plants under field conditions have also been studied, mainly in terms of forage yield (Hodges, Killinger and Carrigan, 1945) and chemical composition (Beeson, Gray and Adams, 1947; Weinmann, 1951).

Apart from their effects on vegetative growth, micronutrients have also been found essential for flowering and seed production of a number of species (Gizzard and Matthews, 1942; Reed, 1942; Hasler and Maurizio, 1947; Wood and Sibly, 1950). This might be a secondary effect, however, since severe deficiencies usually result in cessation of growth, or even death, before the flowering stage.

Nutritional disorders in plants are usually indicated by the development of characteristic symptoms of deficiency or toxicity. Colour atlases of such symptoms in many fruit, vegetable and cereal crops have been compiled (Bear and Coleman, 1949; Wallace, 1951), but information regarding their occurrence in native grasses is relatively meagre.

In addition to visual symptoms, the chemical composition of plants provides en index of their nutritional status; it has often provided the first clue to the atiology of nutritional disorders (Goodall and Gregory, 1947). Both whole plants and specific tissues have been used for this purpose (Goodall and Gregory, 1947).

This report deals with the response of the perennial African grass, *Setaria sphacelata* (Schum.) Stapf and Hubbard (golden millet) to different levels of boron, manganese, copper and zinc. In addition to observations on plant growth and development, symptoms of any nutritional disorders were noted in terms of the supply as well as the internal levels of the elements involved.

MATERIAL AND METHODS

Seeds of *Setaria sphacelata* (Kazungula ecotype, strain P1191) were planted in acid-washed quartz sand in one-litre glazed earthenware crocks. The seedlings were supplied with deionised water for four weeks, and subsequently with Hoagland and Arnon's (1950) No. 2 macronutrient solution, consisting of 0·006M KNO_3, 0·004 M $Ca(NO_3)_2 \cdot 4H_2O$, 0·002 M $MgSO_4 \cdot 7H_2O$ and 0·001 M $(NH_4)_2HPO_4$, buffered at pH 5·5 with H_2SO_4. After two weeks, the plants also received a micronutrient supplement, containing basically 0·5 ppm B, 0·5 ppm Mn, 0·05 ppm Zn, 0·02 ppm Cu and 0·01 ppm Mo. Four series of treatments, in each of which one of the elements boron, manganese, copper or zinc was varied, were initiated at this time. Each series was subdivided into 12 treatment levels, ranging from 0 to 200 ppm, with five plants per treatment.

The plants (one per pot) were arranged in a randomised-block pattern.

Each container was flushed three times weekly with 400 ml of the appropriate nutrient solution, and with 200 ml deionised water containing five ppm ferric tartrate on other days. As the plants grew older, nutrients and water were supplied at more frequent intervals.

For comparative purposes, two plants of each series were also grown in water culture in the absence of the respective elements. One-litre Pyrex beakers were used for the minus Mn, Cu and Zn cultures, and soda-glass quart jars for the minus B culture. Nutrient solution and water were added on alternate days and the solutions were changed weekly. These plants were not analysed for micronutrients.

Nutrient solutions for the boron series were stored in soda-glass containers; Pyrex-glass containers were used for the manganese, copper and zinc series.

For the boron series, reagent-grade chemicals were used without further purification. In the other series stock solutions of the major nutrients were purified according to the method of Steinberg (1935) as modified by Stout and Arnon (1939). Reagent-grade micronutrient salts were used without further purification.

The plants were grown in a greenhouse in Chicago, Illinois, for altogether eight months (from March until November) under conditions of natural day-length. At harvest the shoots were cut off about two cm. above the level of the sand. After removal of the inflorescences (ears), dry weights of the above-ground parts (shoots) were determined after drying at 80°C in a forced-draught oven for 72 hours. Because it was impractical to measure individual tillers, the tillers over and under 150 cm. in length (measured from the point of attachment to the ligule of the youngest leaf) were counted. Tillers over 150 cm. were termed long tillers. The inflorescences were measured, cleaned and their seed production determined.

At the time of harvest, one plant from each series receiving no boron, copper or zinc respectively was left to tiller from the root stock. These cultures were maintained on their respective nutrient solutions until the following spring (April), when each plant was broken up into individual tillers. Five of these were planted in individual pots and given the same minus micronutrient treatment as the original plant, while two tillers from each series were supplied with complete nutrient solutions to serve as controls. All these plants were harvested after four months. Apart from observations on their general development, they were not used for growth or chemical analyses.

For chemical analysis the plant material was ground in a Wiley mill to pass a sixty-mesh screen and dried at 100°C. Boron was determined according to the quinalizarin method (Berger and Truog, 1944), manganese by the periodate method (Cook, 1941), copper according to the carbamate method (Eden and Green, 1940) and zinc by extraction with dithizone (Cowling and Miller, 1941). Colour comparisons were made with a Klett-Summerson photoelectric colorimeter, using the following filters: boron—Corning No. 2412; manganese and zinc—Klett No. 54; and copper—Klett No. 44. All analyses were expressed on an oven-dry basis.

Statistical analyses of the results were carried out according to established methods (Snedecor, 1956).

RESULTS AND DISCUSSION

Visual Symptoms of Nutritional Disturbances:

No definite symptoms of boron deficiency were observed in the main sand-culture experiment. In the accompanying water-culture control without boron as well as in the second minus-boron sand culture, some symptoms of deficiency did develop. The growing points of some tillers of each plant died and elongation of stems and leaf sheaths ceased, resulting in a cluster of leaves at the end of each tiller. These tillers died without flowering. Where ears developed, they failed to emerge completely. The final result was a relatively large number of short tillers. Morris (1931) also observed increased tillering in spring wheat in the absence of boron.

Symptoms of manganese deficiency appeared in plants receiving 0·05 ppm Mn or less. They were most severe in plants receiving no Mn, and appeared first in older tillers shortly before flowering. An interveinal chlorosis developed in the centre of the terminal leaf. This area later became necrotic. Necrosis progressed until the whole leaf as well as the growing point of the stem were dead. Eventually the complete tiller died. In some instances inflorescences developed, but failed to emerge completely before dying. Manganese deficiency symptoms of a similar nature have been observed in *Phleum pratense* (Williams, 1951).

No definite symptoms of copper or zinc deficiency were observed at any time in any of the sand or water cultures.

Symptoms of boron toxicity developed as slight necrotic spots along the margins of older leaves of plants receiving five ppm boron. These spots increased in size as the supply of boron was increased. In addition, the plants receiving 100 and 200 ppm B grew poorly; they had thin stems and were often chlorotic. Some plants receiving 200 ppm B died; none of them flowered.

Symptoms probably caused by manganese toxicity developed only in plants supplied with 100 and 200 ppm Mn. They consisted of small, fairly regular necrotic spots on older leaves and the lower internodes of old tillers; young leaves and tillers appeared quite normal.

Setaria sphacelata appeared to be highly susceptible to copper toxicity. Plants receiving one ppm or more of Cu became chlorotic and their development was retarded. Some of the plants supplied with 5 or 10 ppm of Cu died after a few weeks, those receiving 100 ppm became severely chlorotic and died within two weeks, and application of 200 ppm Cu resulted in the death of all plants after three or four days.

Symptoms of zinc toxicity developed only in plants receiving 100 and 200 ppm of Zn. In addition to severely limited growth, small necrotic spots developed along the midribs of young leaves; large necrotic areas occurred on older leaves. Blackening of lower internodes occurred in plants receiving 200 ppm Zn. Some plants at both these levels of Zn eventually died.

General Developmental Effects:

Emergence of inflorescences and flowering commenced about three months after planting the seeds and continued sporadically among the various series after that. At harvest time only about two-thirds of the plants not definitely inhibited by nutrient treatment had flowered. The lack of floral initiation was not correlated with any particular nutrient treatment, for some plants in most treatments failed to flower. The reason for this failure is not clear.

In addition to total inhibition of flowering several abnormalities in floral development were observed, viz., abortive ears (which consisted mainly of a large cluster of leaves attached to a short, flowerless "spike") and a type of vegetative apomixis (in which vegetative bulbils or plantlets replaced the normal flowers). The latter condition has been noted in a number of grasses, e.g., *Poa*, *Bromus* and *Panicum* spp. (Warmke, 1952). These abnormalities were not related to nutrient treatment, and occurred along with normal ears on the same plants.

During the course of the experiment, attempts were made to determine the effects of nutrient treatments in the substrate upon pollen viability. For this purpose pollen was collected in the morning soon after anthesis and germinated in hanging-drop cultures consisting of 15 per cent sucrose and one ppm B (as boric acid). Because some of the plants did not flower, the results were incomplete. In addition, the temperature in the greenhouse increased very rapidly after sunrise (i.e., at the time the pollen was shed), with the result that the pollen germinated and grew very poorly. Even so, the available results did not indicate any relation between nutrient treatment and pollen viability.

At harvest time, observations as to ear length, seed production per plant, average seed production per ear, and seed production per unit of ear length were made. Again, these results were incomplete because many plants failed to flower. Insofar as could be determined, however, no particular trend or consistency was shown with regard to the effects of any micronutrient element upon any of the abovementioned aspects. The detailed data were regarded as insufficient for inclusion in this report, although the effects of the different treatments upon seed production per plant have been included in the tables.

Effects upon Growth and Chemical Composition:

Boron (Table 1).—In general, the concentration of boron in the shoots was not affected by applications of B up to 0·1 ppm, and only slightly by further increases to 0·5 and 1·0 ppm. Additional increases in the amount of B supplied resulted in significant increases in the B content of the shoots. Total dry weight of the shoots was not affected by B levels up to one ppm, but further increases in the application of B generally resulted in reduced dry weight. The largest number of tillers was obtained with 0·001 ppm B in the medium; 100 and 200 ppm of B resulted in significant decreases in tiller production as compared to the other treatments.

TABLE 1.

Effects of Boron Supply upon Boron Content, Growth, Flowering and Seed Production

Boron Supply ppm	B Content of Shoots mg/Kg Dry Wt	Dry Wt of Shoots g	No. of Tillers per Plant	Per cent Tillers over 150 cm	Per cent Flowering Tillers	No. of Seeds per Plant
0·0	13 ± 2	98·6	57·4	26·7	5·9	28·8
0·001	11 ± 1	96·1	64·4	16·1	4·3	25·6
0·005	13 ± 2	97·3	41·4	40·1	2·0	7·8
0·01	15 ± 1	99·8	48·0	35·5	9·9	40·0
0·05	13 ± 1	96·9	44·8	24·8	2·5	0·8
0·1	15 ± 1	96·1	43·8	36·2	6·3	2·8
0·5	19 ± 2	106·8	45·6	42·5	20·2	13·6
1·0	21 ± 3	103·2	41·2	41·8	17·2	176·8
5·0	69 ± 9	88·1	40·6	32·4	4·9	7·6
10·0	95 ± 10	83·7	37·2	37·2	6·2	19·8
100	998 ± 158	24·3	22·0	31·9	1·4	0
200	1,266 ± 440	2·3	5·4	8·1	0	0
L.S.D. (P = 0·05)		17·9	18·4	16·6	12·2	—
(P = 0·01)		23·9	24·6	22·2	16·4	—

If the percentage of tillers over 150 cm is taken as a measure of tiller length, this aspect of growth was little affected by boron. Significant reductions in the percentage of long tillers were obtained only with 0·001 and 200 ppm of B, which probably corresponded to mild deficiency and toxicity conditions, respectively.

The highest percentages of flowering tillers were obtained with 0·5 and 1·0 ppm of B; above and below these levels flowering was considerably reduced.

With the exception of the 0·5 ppm level of B, some plants of all the treatments failed to flower.

Seed production was extremely variable and showed no consistent relation to treatments, but by far the largest number of seeds were produced by the plants supplied with 1·0 ppm of B. No seeds were produced at the two highest levels of B.

The lack of definite deficiency symptoms, together with the variability of the results (as indicated by the relatively high L.S.D. values) make definite conclusions regarding the effects of boron rather difficult. If one considers the increased tillering obtained in water culture and in the second sand culture in the absence of boron, it seems that a certain degree of boron deficiency might have developed in the plants receiving 0·001 ppm of B. Their internal concentration of B (11 mg per Kg of dry weight) was also the lowest. It seems possible that symptoms of a deficiency of B might develop when the internal concentration of B in *S. sphacelata* should fall below 10 ppm. The optimal level of B application—considering the dry weight of the shoots and the percentage of flowering tillers—appears to be in the vicinity of 0·5 to 1·0 ppm, with an internal concentration of about 20 mg B per Kg of dry material.

Manganese (Table 2).—Applications of Mn up to 0·1 ppm had no effect upon the concentration of Mn in the shoots, but further increases in Mn supply resulted in substantial increases in Mn content. The effects of Mn deficiency are reflected in the dry weights of the shoots, which were significantly reduced at

TABLE 2.

Effects of Manganese Supply upon Manganese Content, Growth, Flowering and Seed Production

Manganese Supply ppm	Mn Content of shoots mg/Kg Dry Wt	Dry Wt of Shoots g	No. of Tillers per Plant	Per cent Tillers over 150 cm	Per cent Flowering Tillers	No. of Seeds per Plant
0·0	10 ± 1	27·4	47·0	5·2	0·0	0·0
0·001	10 ± 0	29·4	42·0	7·2	1·2	5·0
0·005	10 ± 2	44·5	54·8	3·0	3·6	3·4
0·01	8 ± 1	53·4	47·4	7·5	3·8	3·8
0·05	10 ± 0	88·0	58·6	23·4	6·8	17·2
0·1	12 ± 2	97·3	47·0	22·2	17·4	42·4
0·5	36 ± 3	117·0	49·4	31·8	18·3	44·0
1·0	77 ± 7	109·1	48·0	35·5	17·9	62·4
5·0	316 ± 43	128·9	42·6	35·5	12·7	3·8
10·0	515 ± 25	106·7	48·2	42·7	8·1	18·0
100	3,230 ± 249	114·1	50·8	43·6	22·4	94·6
200	3,414 ± 96	81·2	38·8	39·8	10·1	31·0
L.S.D. (P = 0·05)		17·7	18·7	12·0	17·9	—
(P = 0·01)		23·6	25·0	16·0	23·9	—

Mn levels of 0·1 ppm and below. Since these plants developed varying degrees of Mn deficiency symptoms, it seems that such symptoms develop in plants with an internal Mn content of about 10 ppm or less. In general, the best growth was obtained with 5·0 ppm of Mn, and an internal Mn concentration of about 300 mg per Kg dry weight.

S. sphacelata appears to be remarkably tolerant to high levels of Mn. At the 100 ppm level of Mn, the tissues contained more than 3 g of Mn per Kg dry weight, or approximately 360 mg Mn per plant, without growth being affected. The fact that 200 ppm of Mn were supra-optimal is reflected in the reduced dry weight of the shoots.

Although Mn had a pronounced effect upon dry weight, tiller formation was not significantly affected. In comparison to Mn levels of one ppm or above, however, the percentage of long tillers was reduced in plants receiving 0·05 and 0·1 ppm of Mn, and even more so at the lower levels of Mn. This reflects the premature dying of tillers at sub-optimal levels of Mn. Some degree of remobilisation of Mn from older to younger tillers appears to have occurred.

Although flowering (i.e., the percentage of flowering tillers) at levels of 0 to 0·05 ppm of Mn was considerably reduced in comparison to that at 0·1 to 100 ppm, most of these differences were not statistically significant, because of the variation in flowering within each treatment. On the average, only three out of five plants in each treatment flowered; only at the 100 ppm level of Mn did all the plants flower. Seed production, although highly variable, was much lower with 0 to 0·05 ppm of Mn than at most of the levels of Mn above 0·05 ppm.

Copper (Table 3).—The concentration of copper in the shoots was not significantly affected by increases in Cu applications up to 0·5 ppm, but higher levels of Cu resulted in considerable increases in Cu content. The dry weight of the shoots, number of tillers per plant and percentage of long tillers, although varying considerably, were not significantly affected by Cu applications up to 0·5 ppm (and an internal concentration of 8 to 16 mg Cu per Kg dry weight). In the presence of 1·0 ppm of Cu, dry weight and tiller length were somewhat reduced, whereas total tiller production was not affected. These growth effects were associated with an internal Cu concentration of 31 ppm. This level of Cu in the tissues was probably slightly supra-optimal. Further increases in the level of applied copper resulted in significant reductions in all aspects of growth, which were associated with the development of toxicity symptoms of various degrees of severity.

The percentage of flowering tillers remained fairly constant at Cu levels up to 0·05 ppm, but decreased sharply when 0·1 ppm of Cu was applied. All the plants receiving 0·01 and 0·05 ppm of Cu flowered; below these levels, 80%

TABLE 3.

Effects of Copper Supply upon Copper Content, Growth, Flowering and Seed Production

Copper Supply ppm	Cu Content of Shoots mg/Kg Dry Wt	Dry Wt of Shoots g	No. of Tillers per Plant	Per cent Tillers over 150 cm	Per cent Flowering Tillers	No. of Seeds per Plant
0·0	8 ± 2	92·7	37·0	30·8	24·3	56·0
..0·001	8 ± 1	105·9	40·8	38·0	16·2	32·2
0·005	8 ± 1	99·7	36·0	42·4	18·9	15·6
0·01	9 ± 1	111·0	40·6	40·2	19·2	23·4
0·05	10 ± 1	109·5	40·0	37·9	19·5	54·8
0·1	16 ± 4	93·5	46·6	28·1	10·5	58·8
0·5	15 ± 3	103·5	48·4	31·7	11·6	101·2
1·0	31 ± 5	81·5	51·0	23·8	5·9	5·4
5·0	103 ± 32	11·89	17·8	5·2	0·6	0·2
10·0	339 ± 102	1·32	4·2	0	4·0	0·2
100	14,888 ± 2175	0·02	1·0	0	0	0
200	25,549 ± 3288	0·03	1·0	0	0	0
L.S.D. (P = 0·05)		21·3	16·0	15·8	18·1	—
(P = 0·01)		28·4	21·4	21·1	24·2	—

of the plants flowered. Above 0·05 ppm of Cu, the percentage of plants flowering decreased progressively as the application of Cu was increased.

Best seed production was obtained with 0·5 ppm of Cu in the medium; practically no seeds were produced where 5 ppm or more of Cu had been applied.

The results indicate that *S. sphacelata* has a relatively low requirement for copper, with good growth, flowering and seed production at a copper level of only 8 mg per Kg of dry material. It is difficult to single out an optimal level of copper, however. Of the four micronutrient elements tested, the plants appear to be most sensitive (in respect of growth as well as reproduction) to copper, in that applications of 1·0 ppm or more resulted in significant decreases in growth.

Zinc (Table 4).—For some unknown reason the concentration of Zn in plants receiving 0·001 and 0·005 ppm of Zn was lower than in plants not receiving any Zn. Otherwise, the Zn content of the tissues was not appreciably affected by applications of Zn up to 0·5 ppm. Above that level, the Zn content increased markedly as the application was increased.

Although the growth of the plants varied somewhat, applications of Zn from 0 to 10 ppm—and internal Zn concentrations from 9 to about 380 mg per Kg dry weight—had no significant effect upon the dry weight of the shoots or (with one or two exceptions) upon the number of tillers per plant. Both dry weight and number of tillers were considerably reduced in plants receiving 100 ppm or more of Zn, however. Tiller length was rather variable and did not show

TABLE 4.

Effects of Zinc Supply upon Zinc Content, Growth, Flowering and Seed Production

Zinc Supply ppm	Zn Content of Shoots mg/Kg Dry Wt	Dry Wt of Shoots g	No. of Tillers per Plant	Per cent Tillers over 150 cm	Per cent Flowering Tillers	No. of Seeds per Plant
0·0	18 ± 1	114·1	40·0	41·6	8·5	27·8
0·001	12 ± 1	92·4	42·2	13·8	15·9	124·0
0·005	9 ± 1	100·8	34·2	48·2	3·1	1·2
0·01	20 ± 3	104·5	29·0	43·6	16·4	77·6
0·05	23 ± 3	105·3	40·4	29·2	14·2	40·4
0·1	18 ± 1	110·8	45·4	36·4	14·1	27·0
0·5	26 ± 2	94·6	41·2	30·0	22·9	17·4
1·0	48 ± 6	104·4	46·2	34·1	11·5	160·0
5·0	235 ± 31	93·7	45·6	27·7	17·4	150·6
10·0	382 ± 32	105·1	54·2	18·2	15·5	51·4
100	6,533 ±1,883	8·75	9·8	14·2	0	0
200	14,377 ±5,155	2·80	3·6	2·7	3·3	0·2
L.S.D. (P = 0·05)		24·6	14·7	16·3	20·2	—
(P = 0·01)		32·8	19·6	21·8	27·0	—

any consistent relation to treatment or to the internal concentration of Zn; the percentage of long tillers tended to decrease with applications of 10 ppm or more of Zn.

The effects of Zn upon flowering were also extremely variable and most differences were not statistically significant. The lowest percentage of flowering tillers per plant were obtained with 0·005, 100 and 200 ppm of Zn. About 60% of the plants in each treatment up to 10 ppm of Zn flowered; only with 0·5 ppm of Zn did all the plants flower.

Seed production also varied considerably and showed no consistent relation to treatment, except that practically no seeds were produced by plants supplied with 100 and 200 ppm of Zn. Best seed production was obtained with 1 and 5 ppm of Zn.

From the results it would seem that a deficiency of Zn would only develop in plants with an internal level of Zn of less than 9 mg per Kg dry weight, and that *S. sphacelata* is able to accumulate fairly large amounts of Zn (up to about 400 mg per Kg dry tissue) without apparent injury or retardation of growth. Although it is not possible to define an optimal level of Zn application, amounts above 10 ppm in the medium would appear to be supra-optimal.

CONCLUSIONS

Of the four micronutrient elements investigated, only manganese was definitely shown to be required for normal growth and development of *Setaria sphacelata* in sand culture. Not only did the plants show definite symptoms of deficiency in the absence of Mn, but growth was greatly improved and the deficiency symptoms were absent at Mn levels of 0·5 ppm and above. In addition, the plants appear to be more tolerant to high levels of Mn than to comparable levels of any of the other three elements.

Although no definite symptoms of a deficiency of boron developed in the main sand culture experiment, boron also appears to be required for normal growth and development. This was indicated by the development of some symptoms of disturbed growth in the minus-boron plants during the accompanying water-culture and the second sand-culture experiments. It is also possible that the increased tillering, coupled with the reduction in tiller length observed in those plants receiving 0·001 ppm of B in the main sand-culture experiment, might indicate the development of an incipient deficiency of boron.

The absence of deficiency symptoms or specific growth effects in those plants receiving little or no Cu or Zn, together with the fact that some of them contained as little as 8 mg Cu and 9 mg Zn respectively per Kg of dry material, seem to indicate that *S. sphacelata* has a relatively low requirement for these elements, as compared to B and Mn. Analysis of the original seed showed it to contain 19 mg B, 102 mg Mn, 355 mg Cu and 324 mg Zn per Kg of dry matter. Since the seeds weigh about one milligram apiece, it appears that contamination from outside sources had not been eliminated completely.

In view of the results obtained, one would expect deficiencies of Cu and Zn to be less likely than those of Mn (or even of B) under field conditions. On the other hand, the sensitivity of the plants to Cu levels as low as one ppm would indicate that this element might have deleterious effects on their growth under certain circumstances.

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